Biochemical and Toxicological Response of Infant Baboons to Lead Driers in Paint

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In an effort to define the toxicology and disposition of lead compounds that presently exist in paint (i.e., organic driers), a controlled dose feeding study was initiated early this year with the use of 28 infant baboons as experimental animals. The infant baboon, established as a metabolic model for a child ingesting lead, will be used to determine the adequacy of present as well as recently recommended limitations for lead in paint to assure protection from this potential source of lead exposure.

To accomplish this goal, research has been designed to determine basic dose–response relationships in animals ingesting constant daily doses of a dried paint, a lead octoate drier, and lead acetate. Doses for these compounds have been chosen to cover a broad range of concentrations including that recommended by the American Academy of Pediatrics from the maximum daily permissible lead ingestion, and associated estimates of paint intake by children with pica.

Parameters of metabolic response for each lead compound, include: general clinical surveillance, lead concentrations in blood, urine and feces, erythrocytic δ-aminolevulinic acid dehydratase and free erythrocytic porphyrin. The response of several of these measures of lead exposure as a function of time will be discussed for each compound at the several dose levels administered.

Introduction

As a result of the high incidence of lead poisoning observed in children who ingest dried paint that was manufactured during the early 1940's, lead pigments have virtually been eliminated from modern consumer paints. The older paints commonly asserted to cause intoxication may contain as much as 50–60% lead by weight in their dried solids. However, there is still some question as to whether the present limit of 0.5% lead in dried paint solids will assure complete safety in children with pica. It has been recommended, therefore, that permissible levels of lead in consumer paint be reduced to 0.06% of the dried solids (*1*) which would, of course, be somewhat lower in the actual liquid paint formulation; this recommendation is currently under consideration. To meet this requirement, it would be necessary to eliminate further the remaining lead additives in paint, i.e., substitution of nonlead compounds for driers such as lead octoate (lead 2-ethylhexoate).

The suggested limit of 0.06% was based largely upon data (and the extrapolation of data) derived from studies which have defined the toxicity of lead in chemically solu-

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ble forms rather than from tests on actual paint formulations. The present investigation is concerned with the toxicology and disposition of one lead compound, lead octoate, that presently exists in consumer paint, as compared to other and more soluble lead compounds. These studies have been performed in the infant baboon, a primate whose susceptibility to lead encephalopathy has now been established (*2–5*). As an experimental model for the child ingesting lead in paint, dose-response relationships in the baboon will provide one means of determining the adequacy of present and recommended regulations for lead in paint. The research program has been designed accordingly to determine the basic effects of lead ingestion in infant baboons ingesting constant daily doses of dry paint, a lead octoate drier, or lead acetate. Doses for these compounds have been chosen to cover a range of concentrations to include that recommendation by an *ad hoc* committee of the American Academy of Pediatrics for the maximum daily permissible lead ingestion (*6*) and associated estimates of paint intake by children with pica (*1*).

Parameters of metabolic response examined for each lead compound include: general clinical surveillance, lead concentrations in blood, urine and feces, erythrocytic δ-aminolevulinic acid dehydratase (ALA–D) and free erythrocytic porphyrin (FEP). The response of each of these measures of lead exposure as a function of time will be discussed for each compound at the several dose levels administered.

Methods and Materials

As the present study is a range-finding effort, only two animals have been exposed at most dose rates. The dose regimen was chosen to cover a range of concentrations that would represent the daily ingestion of 1 in.^{2} of indoor paint six layers thick (6.5 mg/cm^2/layer) at the present (0.5%) and recommended (0.06%) limits, as well as at levels two and five times the 0.5% regulation. Paint containing the lead octoate drier was supplied through the courtesy of the Sherwin-Williams Co., and specially prepared samples of lead octoate were provided by Cincinnati Milacron Chemical, Inc. and Tenneco Chemicals, Inc.

To compare the effects of lead in different compounds of varying solubility, the same doses were repeated in other animals with either lead octoate drier in olive oil or lead acetate. In two animals a higer dose was administered intravenously as lead chloride to demonstrate the effects of high concentrations of lead in the blood. The complete dosage schedule is given in Table 1. In all cases of ingestion, lead compounds were administered daily in single gelatin capsules at approximately the same time of day. All paint samples were ground and passed through a 0.5 mm sieve (No. 35 Standard). This particular particle size has been chosen as a conservative estimate for considerations of gastrointestinal absorption.

Dose-response and dose rate-response relationships are studied by monitoring lead concentrations in blood, urine and feces, as well as the levels and activities of compounds associated with heme synthesis, specifically FEP and ALA–D. Brief descriptions of the analytical techniques follow.

Blood Lead Concentrations

Measurement of lead in whole blood was determined by atomic absorption spectrophotometry by using the Cernik modification of the Delves cup microsampling technique (*7*). Basically this technique involves the assay of 10 μl of sonicated whole blood which has been spotted and dried on filter paper and subsequently measured with an Instrumentation Laboratories, Inc. atomic absorption spectrophotometer by using a modified Jarrell-Ash Delves cup apparatus. The lower limit of detection by this method is 3 μg of lead per 100 cc whole blood (3 μg-%) at the 95% confidence level. The x-ray fluorescence determination of lead in dried compressed blood (G. Laurer and T. J. Kneip, unpublished data) and methyl isobutyl ketone (MIBK) extraction of lead in blood (*8*) are two alternative techniques that were employed for verification of the Delves cup technique.

Table 1. Lead compound dose rate schedule.

Dose rate, μg Pb/kg-day [a]	No. baboons in group	Compound administered	Equivalent % Pb in paint [b]
12	2	Dried paint solids containing 0.5% lead as lead octoate	0.06
100	4		0.5
200	2		1.0
500	2		2.5
100	2	Lead acetate	0.5
200	2		1.0
500	2		2.5
10000	2		50.0
100	2	Lead octoate in olive oil	0.5
500	2		2.5
1610	2	Lead chloride (IV)	—
Controls	4	Empty gelatin capsules or sodium bicarbonate	—

[a] Baboons' weights range from 2 to 5 kg. Estimated age 4–8 months; equivalent human age, 1–2 years.
[b] Based on intake of 1 in.^{2} of six layers of paint having an estimated solids content of 6.5 mg/cm^2/layer.

Erythrocytic Pophyrin Concentration

Erythrocytic porphyrin concentration was determined by fluorometric techniques (*9*) on whole blood which has been spotted and dried on filter paper (S. Piomelli, unpublished data). Interlaboratory comparison of early results (S. Piomelli, and P. Young, personal communication), has provided knowledge of refinements which have further minimized test interferences.

ALA-D Activity

δ-Aminolevulinic acid dehydratase activity was determined in hemolyzed blood by the method of Granick (*10*) with modifications suggested by the author (J. L. Granick, personal communication). As a result of variation and early inconsistencies in ALA–D test results, specific and detailed efforts were made to define the source of suspected analytical interferences. From these studies, it was determined that the problem resulted from either contact between the blood and the rubber stopper or activation by some agent associated with the "minimal-lead" glass collection tubes obtained from Becton-Dickinson and Company and certified to contain not more than 0.1 μg Pb/tube (T. Kneip, N. Cohen, and V. Rulon, unpublished data). Sample collection was subsequently performed in polystyrene tubes without rubber stoppers.

Work in progress includes studies of the accuracy and precision of methods used in the analysis of tissue, feces and urine lead contents. Tissue specimens removed at autopsy were frozen for future analysis. In all cases of animals dying as a result of lead poisoning, brain tissue was immediately stored in formalin for pathological examination. Insufficient data precludes discussion of absorption-retention values or lead tissue burdens at the present time.

Results

At the very outset it should be re-emphasized that the present paper refers to work still in progress. Therefore, interpretation of preliminary data is subject to revision as more information becomes available.

Baseline or "normal values" for all measures of lead exposure were established for each animal during a 2-week period prior to the initiation of the exposure program. In addition, four baboons were fed gelatin capsules either empty or containing sodium bicarbonate and are being sampled at the

same rate as the most frequently sampled test animals. (The dose of sodium bicarbonate administered was chosen to simulate the basicity of a lead hydroxycarbonate compound now being administered in parallel ingestion studies involving the toxicity of high levels of this pigment.) The blood lead, FEP, ALA-D, and hematocrit data obtained from two of these control baboons are given in Figure 1. Blood lead concentrations for these animals remained below 20 μg/100 ml and were for the most part, no higher than 15 μg/100 ml. Baseline blood lead concentrations less than these values have been observed for some of the animals in this study and could be related to the length of time that the animal or the animal's mother was in captivity on a commercial laboratory diet. [Estimated lead intake from food is derived from Purina Biscuit Chow lead content of 1 ppm and a food intake equaling approximately 4% of the body weight per day. For a 2-kg animal ingesting approximately 80 g food/day, this would amount to a daily food lead intake of 80 μg/day (~40 μg/kg-day].

In general, baboons captured "in the wild" had lower mean blood lead concentrations,

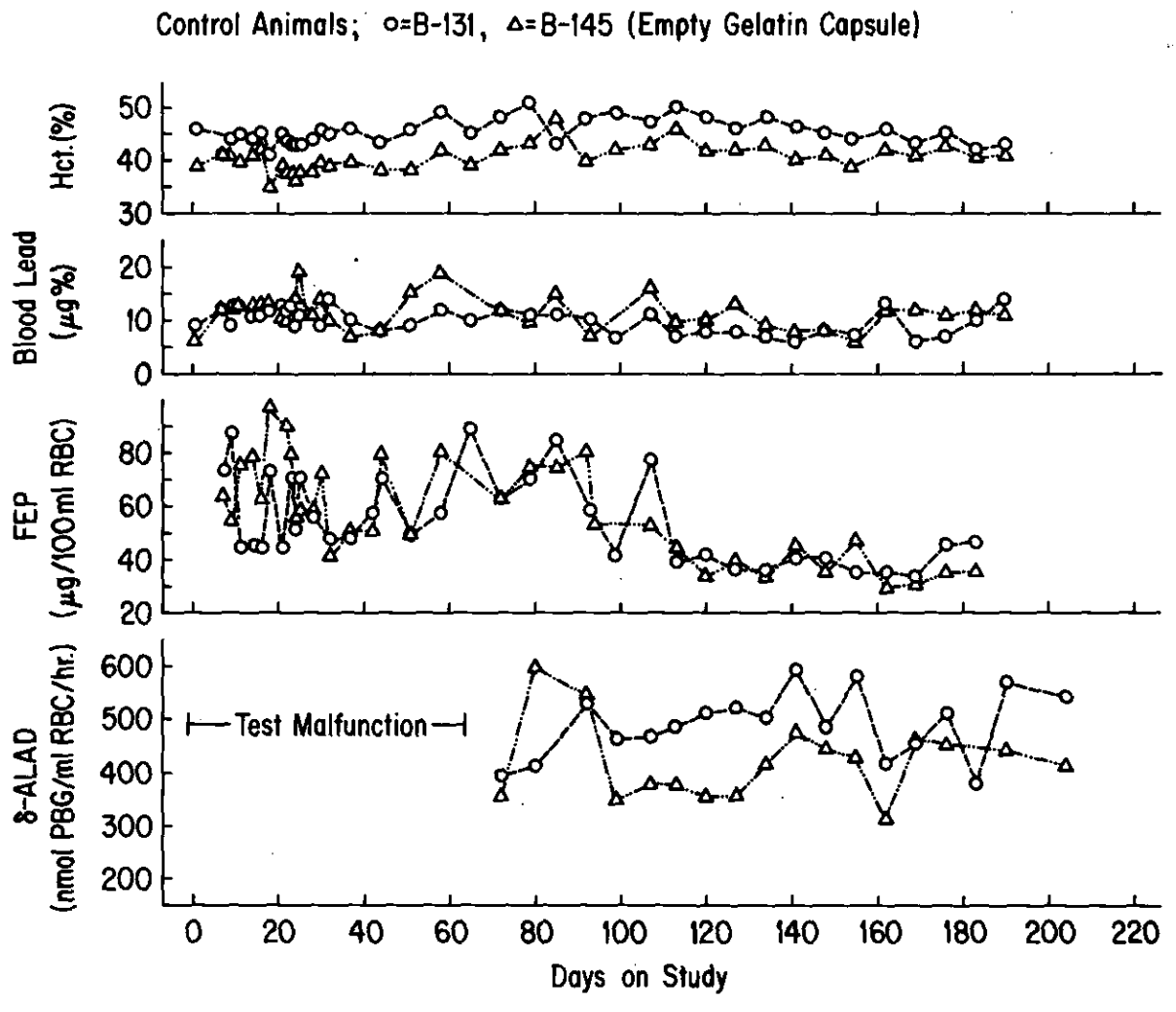

FIGURE 1. Baseline values for two control baboons ingesting empty gelatin capsules; baboons B-131, B-145.

X = 7.0 ±3.0 μg/100 *ml* than animals born or weaned in captivity X = 21 ±3.0 μg/100 ml. It is important, therefore, in considering changes in this parameter as a function of lead dose administered, to use an appropriate baseline or control value taking into account the animal's early background before arriving at the primate colony. Porphyrin concentrations ranged from 30 to 90 μg FEP/100 ml RBC with greater precision (range equals 30 to 50 μg FEP/100 ml) becoming evident midway through the measurement period due, presumably, to a change in the reagent grade made at this time.

Problems with the ALA–D assay, as described earlier, occurred in the early stages of these studies and are indicated in Figure 1 and other figures as "test malfunction" whenever the data for a particular test period were suspect. Once the difficulty was resolved, normal values of ALA–D activity were seen to range from 350 to 600 nmole PBG/ml RBC-hr. It is of further interest to note a distinct and almost constant difference between the two control animals, B–131 having ALA–D values a factor of approximately 1.3 times greater than those for B–145. A difference of similar magnitude is noted for measurements of respective hematocrit values which, according to the calculation for ALA–D, should normalize values for differences in RBC concentration. The FEP background values are both within the ranges reported as "normal" for a child.

For purposes of demonstrating the immediate response of these exposure indicators when a soluble lead compound is injected directly into the blood, two animals were given IV injections of 1.61 mg of lead per day as lead chloride. The dose-response relationships obtained are shown in Figure 2. For these animals the effects of lead are easily observed, i.e., the blood lead concentration increases immediately after the first injection and continues to rise until the animal's death. For the case of animal B–161, the final blood lead concentration before death (at 68 days) was greater than 1500 μg/100 ml while for baboon B–157 the blood lead concentration was approximately 950 μg/100 ml before death (at 85 days). In both cases there is some indication that the increase was not linear as a function of time with the more slowly increasing component becoming apparent one month after the start of exposure.

Porphyrin elevation, however, was not apparent until about 16 days after the initial injection, and had approached a level of 250 μg FEP/100 ml of RBC [considered indicative of increased lead absorption (*9*)] after approximately 1 month. Immediately preceding death, porphyrin levels rose to almost 300 and 500 μg FEP/100 ml of RBC for B–161 and B–157, respectively.

Unfortunately, there was considerable variation in the ALA–D analysis during this period thereby rendering data questionable for this parameter.

Both animals receiving the IV lead chloride injections died within 3 months of the initial injection. Each had recurrent generalized myoclonic convulsions and became blind for a period of 2 to 5 days prior to death. Pathological examination of brain tissue was diagnosed as indicative of encephalopathic disorder (I. Feigin and R. A. Clasen, personal communication).

Due to space limitations, only the results for those baboons ingesting 100 and 500 μg Pb/kg-day, corresponding to 0.5 and 2.5% lead in dried paint solids, respectively, will be considered. Since animals receiving a dose rate of 12 μg Pb/kg-day are ingesting an amount of lead that falls within the expected range of variability for their daily lead intake from food, the data were inconclusive and did not demonstrate any change from pre-exposure levels after 8–9 months from the start of ingestion. It should be emphasized, however, that for small chronic doses such as 12 μg Pb/kg-day, time may be a critical factor. The effects of low levels of lead administered chronically may become manifest only after considerably longer periods of exposure. Results obtained for those animals ingesting dried paint solids and lead acetate at the 0.5% equivalent dose rate of 100 μg Pb/kg-day are presented in Figures

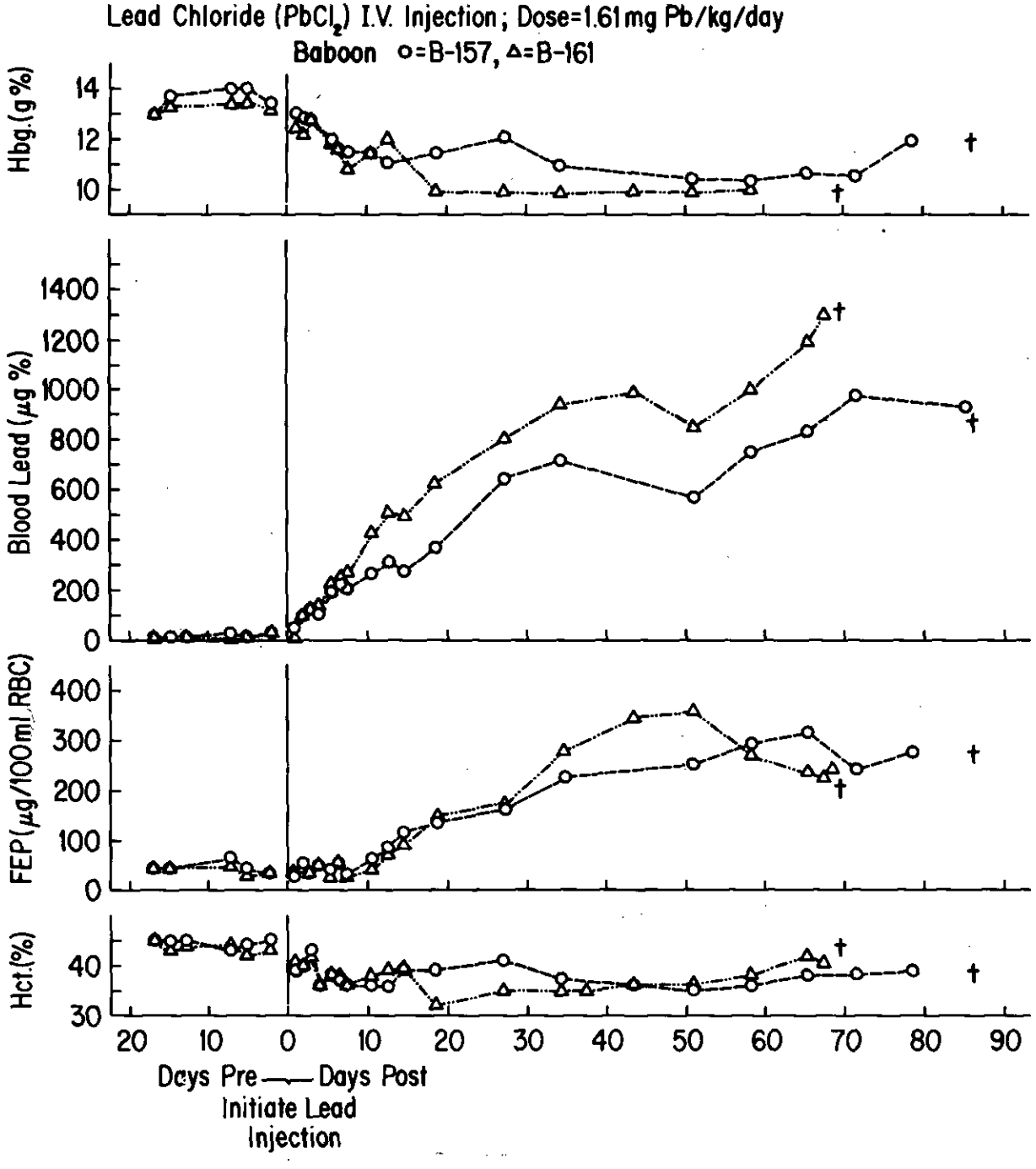

FIGURE 2. FEP and blood lead concentration time-related response to lead chloride administered IV at a dose rate of 1.61 mg Pb/kg-day; baboons B–157, B–161.

3 and 4. In the case of animals ingesting lead in paint, the mean blood lead concentration of 12.2±0.5 μg/100 ml is statistically greater than control values of 9.9±0.5 μg/100 ml. For lead acetate ingestion at this dose rate, an upward trend in blood lead concentration is evident after only 1–2 months of exposure. Likewise, ALA–D levels have been decreasing more rapidly for the acetate ingestion than for the drier in paint compound over the same time period. FEP concentrations have not changed significantly from baseline values in either case at this dose rate.

The results obtained at the 500 μg Pb/kg-day dose level for lead octoate in paint and those for lead acetate at the same dose rate indicate a significant increase of the blood lead concentration regardless of whether baseline or different control animal values are considered. It can be seen from Figures 5 and 6 that the net increase for the lead acetate was greater than for the lead octoate in paint. Concomitant with the blood lead increases was an apparent although small drop in the respective values of ALA–D for all four animals. Once again this depression was somewhat more evident for the animals ingesting lead acetate than for those given dried lead paint. Data for

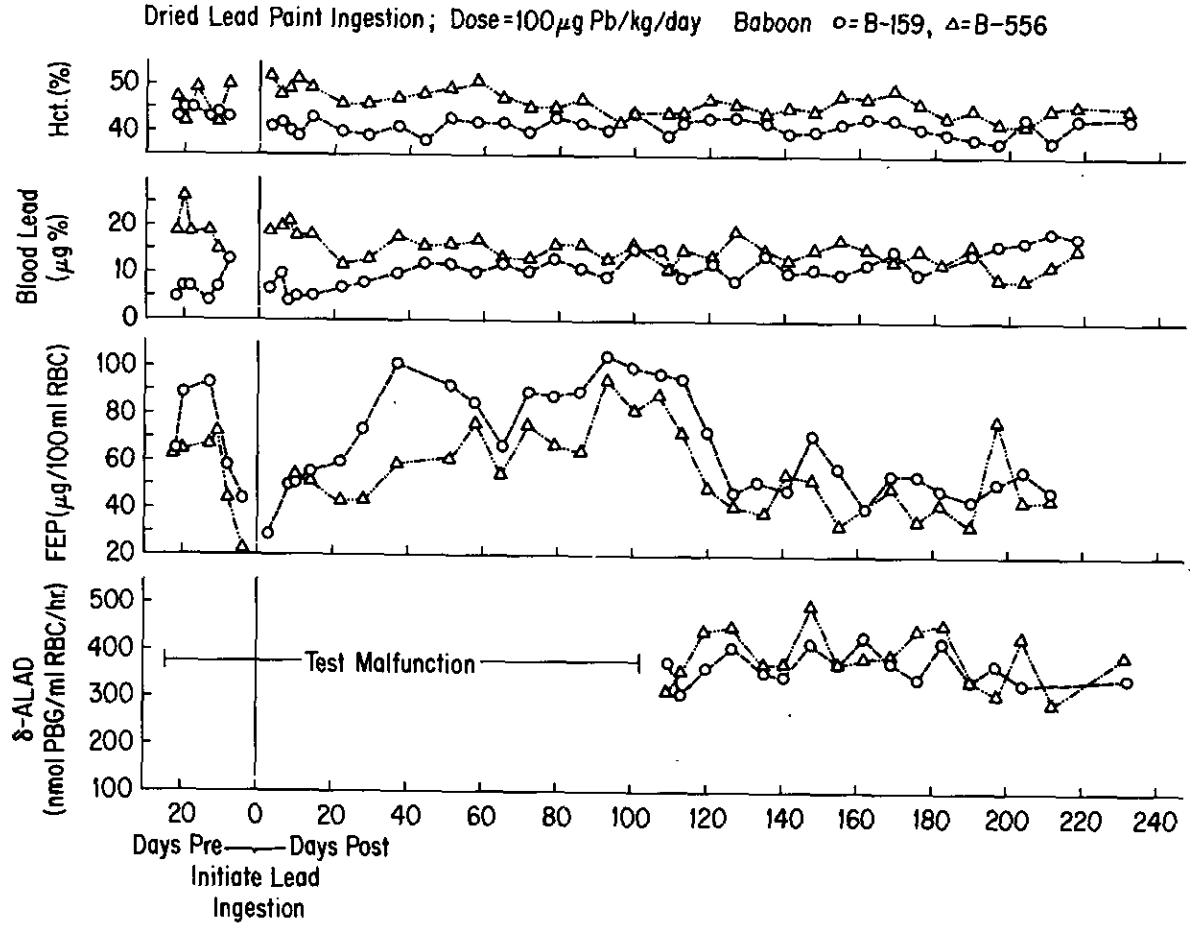

FIGURE 3. ALA–D, FEP, and blood lead concentration time-related response to dried paint containing octoate drier. Exposure by ingestion at a dose rate of 100 μg Pb/kg-day; baboons B–159, B–556.

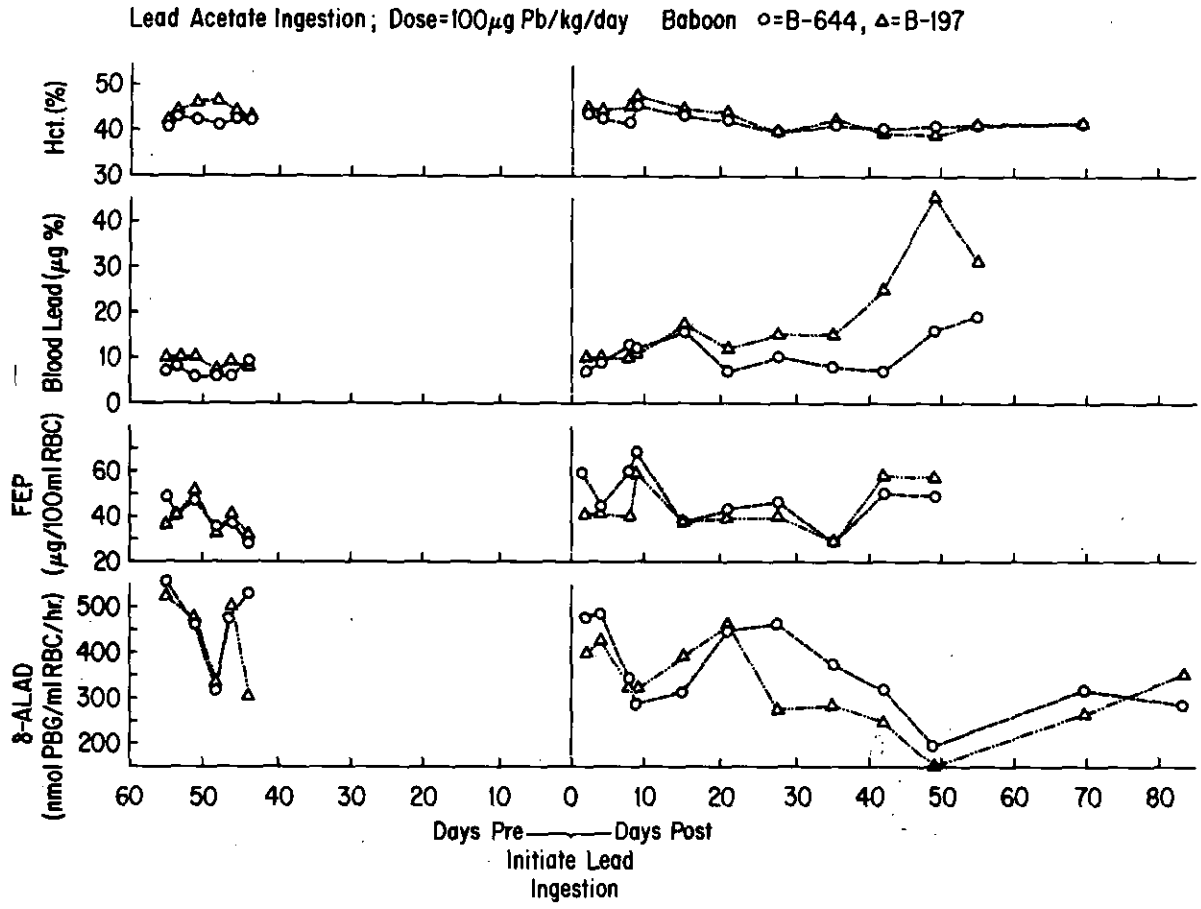

FIGURE 4. ALA-D, FEP, and blood lead concentration time-related response to lead acetate. Exposure by ingestion at a dose rate of 100 μg Pb/kg-day; baboons B-644, B-197.

ALA-D presented in the figures for the time period bracketed by arrows are uncertain, due to test variation. Concentrations of FEP in both cases did not appear significantly different from control values.

The present study includes the investigation of the pure octoate compound administered in olive oil for comparison of the results to those obtained with the dried paint. For this compound, there was an almost immediate increase in blood lead concentration with a corresponding decrease in ALA-D activity at both the 100 and 500 μg Pb/kg-day dose levels. Since this phase of the study is relatively short term at present, the preliminary data collected over the first 9-day ingestion period are presented in tabular form (Table 2).

Discussion

ALA-D

The ALA-D test has proven extremely difficult to control at the early stages of this study. Not only is this enzyme extremely sensitive to temperature but, as previously discussed, there is the problem of exogenous SH groups or other chelating agents that may be present at any stage in the analysis. Since significant genetic variation can be expected for normal ALA-D values even among members of the same species

Table 2. Preliminary response to ingestion of lead octoate drier in olive oil.

Baboon number	Dose rate, μg/kg-day	Blood, lead, μg%	ALA-D, nmole PBG / ml RBC/hr	Hct, %	Hgb, g-%
B–656	Pre-exposure [a]	8.0	386	44	12.8
B–656	100 [b]	21.0	163	41	11.6
B–191	Pre-exposure	8.0	612	43	12.3
B–191	500	24.0	175	37	11.1
B–654	Pre-exposure	11.0	419	43	13
B–654	500	28.0	146	44	12.8

[a] All pre-exposure values are the means of six determinations made during the 2-week period immediately preceding the start of lead ingestion.

[b] Values represent the means of data accumulated during the 9-day period following the start of ingestion.

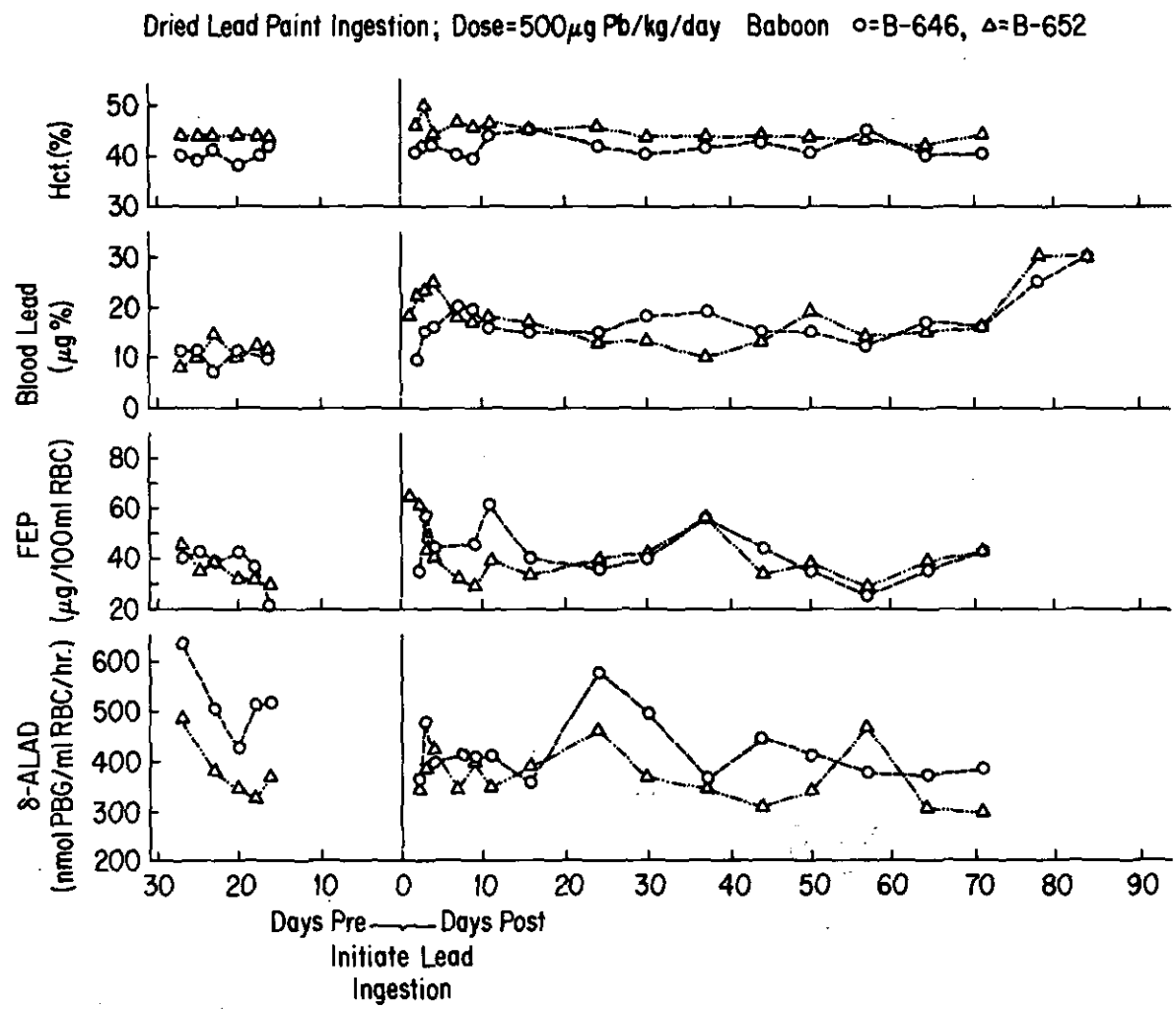

FIGURE 5. ALA–D, FEP, and blood lead concentration time-related response to dried paint containing octoate drier. Exposure by ingestion at a dose rate of 500 μg Pb/kg-day; baboons B–646, B–652.

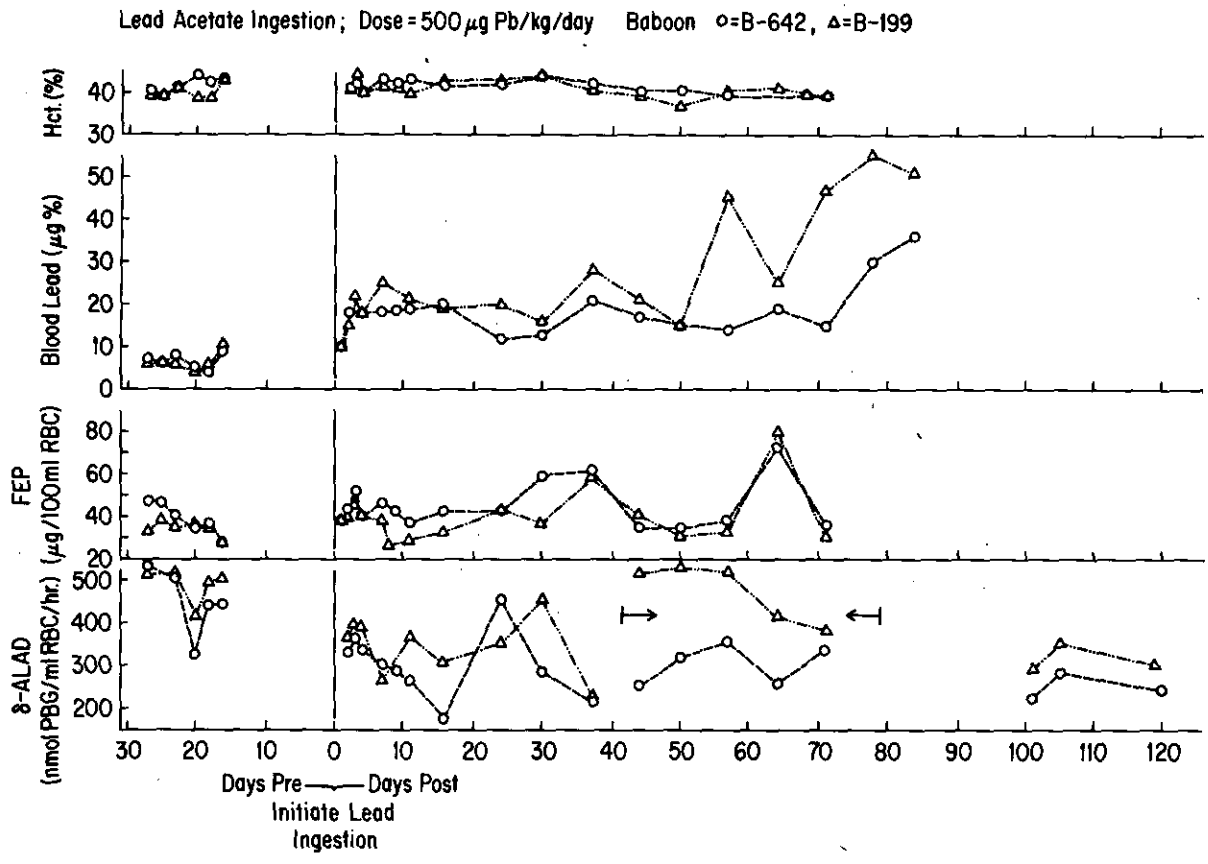

FIGURE 6. ALA–D, FEP, and blood lead concentration time-related response to lead acetate. Exposure by ingestion at a dose rate of 500 μg Pb/kg-day; baboons B–642, B–199.

(*11*), it is preferable to use internal or pre-exposure controls as a baseline for comparisons of these effects of lead ingestion.

Normal values of ALA–D for the infant baboon are generally in the range of 380 ±90 nmole PBG/ml RBC/hr as opposed to 1,000±290 nmole PBG/ml RBC-hr for children (*11*). Depression of normal ALA–D levels to values to 150 nmole PBG/ml RBC-hr or less occurred within two days after administration of either 100 or 500 μg Pb/kg-day as lead octoate in oil and to values of about 300 nmole PBG/ml RBC/hr for lead in dried paint at the same dose rates. Dramatic and immediate depression was noted in those animals administered higher doses of lead acetate (Fig. 7). The ALA–D test appears then as an adequate early indicator of lead exposure at all of the dose levels considered except perhaps at 12 μg/kg-day. The significance of these depressions in terms of biological damage or toxicity still remains speculative at the present time.

Porphyrin Assay

Since porphyrin concentration is elevated not only in lead poisoning, but also in iron deficiency anemia, all animals were given supplemental iron (Inferon dose 50 mg Fe administered IM over a 2-day period at least one week prior to routine blood sampling) before the start of the exposure period. Early analytical difficulties with the porphyrin test were solved by changing from "reagent" to "spectrophotometric" grade

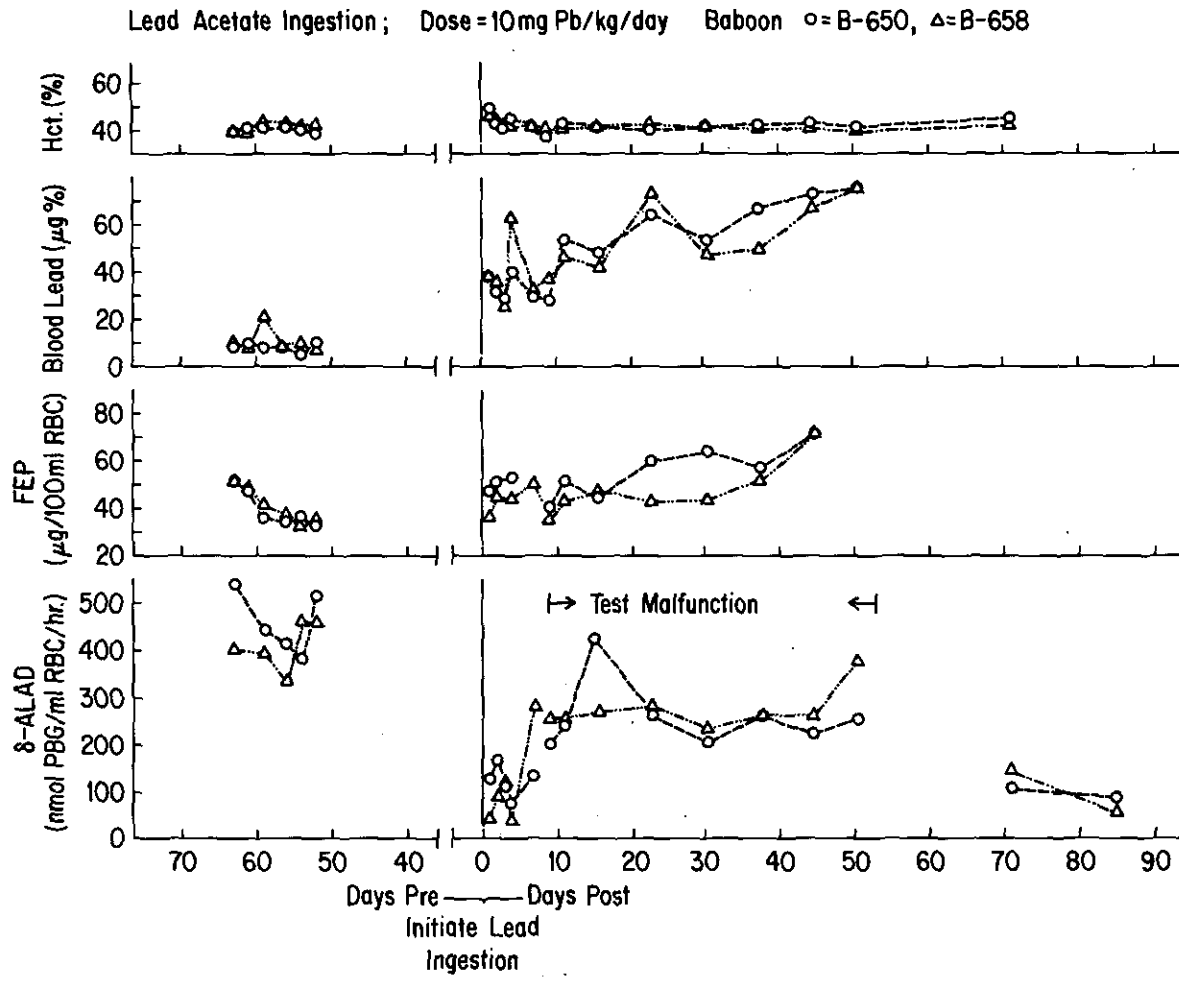

FIGURE 7. ALA-D, FEP, and blood lead concentration time-related response to lead acetate. Exposure by ingestion at a dose rate of 10 mg/kg-day; baboons B-650, B658.

reagents. Blood porphyrin levels are believed to reflect the concentration of lead in the bone marrow existing 2 weeks to 3 months prior to sampling rather than the circulating blood lead level (*12*). The delay in FEP response noted in these studies supports the theory that some tissue burden must be present prior to an increase in FEP. This effect is particularly apparent in the case of those animals injected with 1.61 mg Pb/kg-day as lead chloride or ingesting 10 mg Pb/kg-day as lead acetate where it can be seen that a response is not observable until shortly after the first week of lead exposure. There has been no readily observable alteration of FEP level for any of the animals fed 100 to 500 μg Pb/kg-day either as the acetate or as dried paint. It is reasonable to assume, however, that FEP changes may become more evident as the study progresses. Normal values for the infant baboon were in the range of 30 to 55 μg FEP/100 ml RBC ($X = 46 \pm 10$) as compared to approximately 30–50 μg FEP/100 ml noted for children (*12*).

Blood Lead Concentration

Finally, it has been determined that, except for those animals ingesting 12 μg Pb/kg-day, all of the doses described resulted in an increase in the mean blood lead concentration. The magnitude of this increase as a function of the daily lead intake has been plotted in Figure 8, where it can be seen that increases above normal, ranging from approximately 2 to 6 μg/100 ml, result from ingestion of dried paint at 100 to 500 μg Pb/kg-day, respectively. The increase for lead administered as lead acetate at 100 μg Pb/kg-day is similar to that for the paint at this dose, while there appears to be a somewhat greater increase (approximately 9 μg/100 ml) for lead acetate fed at the 500 μg/kg-day dose as compared to the lead paint results. These increases appeared early for both compounds, within 5 days at the higher dose levels and somewhat more gradually at the 100 μg/kg-day dose. Unfortunately,

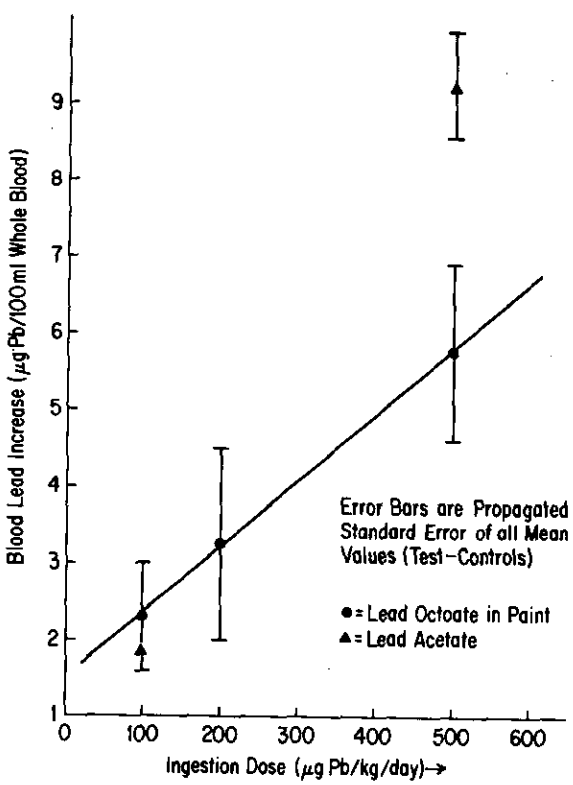

FIGURE 8. Net increase in blood lead concentration after 2–5 months of daily ingestion of lead octoate drier (in paint) and lead acetate. See Table 3.

Table 3. Net increase in blood lead concentration after 2–5 months of daily ingestion of lead octoate drier (in paint) and lead acetate.

Ingestion dose in dried paint, μg Pb/kg-day	No. of animals on test	No. of Controls	Months on test
100	4	2	5
200	2	2	2
500	2	2	2

blood lead data for the 12 μg/kg-day dose have been quite variable, and results are inconclusive to date. At the present time the blood lead values for both doses of acetate (100 and 500 μg Pb/kg-day) and for the higher dose of paint, have begun an upwards trend, while those for the lower doses of paint are remaining relatively constant.

Summary

Significant early results of the present study can be summarized as follows.

Blood lead concentrations have been increasing during the exposure period and were significantly greater than control values for dose rates of 100, 200, and 500 μg Pb as dried paint per kg per day. An upward trend has become evident for doses of lead as lead acetate after approximately 2–3 months from the start of ingestion. A similar trend is observable for paint only at the 500 μg Pb/kg-day dose.

The ALA–D test responds rapidly to lead acetate exposure at dose rates of 500 μg Pb/kg-day and greater. Depression of ALA–D activity at 100 μg Pb/kg-day (as lead acetate) occurred after somewhat longer times from the start of ingestion.

The FEP test responds more slowly to lead exposure than either of the above parameters and is believed to be a better reflection of the total lead exposure than is the circulating blood lead.

Results from animals ingesting similar doses of lead as dried paint solids or as lead acetate are indicative of the greater solubility of the acetate compound.

Early data from animals ingesting lead octoate compound in olive oil show an immediate increase in blood lead concentration

along with a corresponding depression of ALA–D activity.

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